Multimodal action of Mas activation for systemic cancer cachexia therapy

Cancer cachexia remains a largely intractable, deadly condition for patients with no approved, effective therapies. However, research progress over the past few decades demonstrates that cachexia is a disease with specific, targetable mechanisms. New work by Murphy and colleagues in this issue of *Cancer Research* suggests that activation of the alternative renin-angiotensin system with the non-peptide Mas receptor agonist AVE0991 holds promise for reducing muscle wasting in cancer. Their cell studies demonstrate on-target activity in skeletal muscle cells, while their mouse results suggest potentially more important systemic effects.

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Cachexia, extreme weight loss and muscle wasting, is most readily recognized when patients with late-stage cancer become disturbingly emaciated prior to death, but in reality, adipose and muscle loss are often detectable in the early stages of disease. Moreover, cachexia is often underdiagnosed and the magnitude of muscle loss under-appreciated due to the predominance of obesity, particularly among cancer patients in Western nations. Using quantitative imaging analysis, it is estimated the incidence of cachexia ranges from 15% to 85% across tumor types (1). However, regardless of tumor type, the majority of patients with late-stage disease suffer cachexia and some 25-30% of cancer patients are thought to die of cachexia, rather than other effects of the tumor (1). The low muscle mass, systemic inflammation, and dysmetabolism characteristic of cachexia also impair treatment response to surgery, radiation therapy, and chemotherapy. Inadequate to its impact, from 2004-2018 only 20 pharmacological clinical trials have been reported in cancer cachexia (2). Work over the past several decades has vastly increased the knowledge around the physiological, cellular, and molecular interactions leading to adipose and muscle wasting in cachexia, demonstrating that cachexia is not an inevitable consequence of cancer, but rather a disease condition with specific, targetable mechanisms.

Tumors create metabolic and immune havoc, hijacking the body's normal response to injury and inflammation. Cancer cells activate host defenses causing anorexia, malabsorption, reduced activity, asthenia, inefficient energy utilization, abnormalities in the innate and adaptive immune systems, and often persistent systemic inflammation. Such phenomena lead to progressive weight loss, regardless of conventional nutritional support. Thus, cachexia is a syndrome with multiple affected organ systems and a characteristic spectrum of symptoms (3). Nevertheless, consensus definitions of cancer cachexia specify degree of weight loss and/or skeletal muscle depletion as diagnostic criteria (4). Most cachexia research has focused on skeletal muscle wasting, due to its central role in mobility, function, respiration and metabolism, and due to the association of low muscle mass with mortality. Indeed, the

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systemic effects of cancer ultimately produce muscle wasting through impaired anabolism, protein degradation, metabolic dysfunction, and blunted myogenesis. To date, specific interventions to increase muscle mass or block muscle wasting have prolonged life and function in mouse models of cancer cachexia, suggesting that saving muscle could improve survival and outcomes for patients with cachexia (1).

Work by Murphy and colleagues in this issue of *Cancer Research* opens a new line of investigation by interrogating the alternative arm of the renin-angiotensin system (RAS) in cancer cachexia (5). The RAS system was originally identified as a regulator of blood pressure and fluid balance but is now known to play a variety of roles in health and disease in multiple organ systems, including the brain, kidney, heart, adipose tissue, and skeletal muscle (6). Angiotensinogen is cleaved by renin to angiotensin I, and subsequently by angiotensin converting enzyme (ACE) to form angiotensin (Ang) II, which activates type 1 or type 2 receptors (AT1R, AT2R). Ang II signaling increases sympathetic outflow and vasoconstriction, but also promotes fibrosis and oxidative stress. Ang-(1-7) can be generated from Ang I or Ang II by ACE2, and activates Mas (gene name *Mas1*), a member of the G protein-coupled receptor family. Ang-(1-7) and Mas generally oppose activities of Ang II and AT1 and AT2 receptors (6). Specificity in regulation of this pathway is provided in part by special and temporal differences in receptor and enzyme distribution. In skeletal muscle, it is well-established that Ang II signaling on AT1 can induce atrophy and fibrosis (7). Antagonism of this pathway either through Ang II inhibition or ACE2/Ang-(1-7)/Mas activation can lessen muscle atrophy and dysfunction in muscular dystrophy, disuse atrophy, and sepsis models (7).

Herein, Murphy and colleagues show that Mas receptor expression is decreased in the muscle of mice and patients with cancer cachexia, while AT1R is increased. This result suggests a context of active classical RAS signaling and repressed alternative Ang-(1-7) signaling in cachexia. Furthermore, they show that in cultured muscle cells, Mas expression, Ang-(1-7) or the non-peptide Mas receptor agonist AVE0991 can block atrophy induced by serum starvation or by co-culture with cachexia-causing cancer cells, although neither has a hypertrophic effect in normal culture conditions. These results indicate that Mas activation can counter atrophy-promoting pathways directly on muscle cells. Some novel proteomic and miRNA studies included here point to potential muscle-specific mechanisms for further investigation. Finally, they show convincingly that AVE0991 can protect mice from cachexia induced by C26 colon carcinoma cells, demonstrated by the reduction in weight loss and muscle wasting as well as anorexia and activity decline that accompanies tumor growth in this model.

Cachexia is a systemic condition assayed in mice, and the Murphy and colleagues study is a pharmacological study rather than one that employs muscle-specific genetic manipulation of the Mas pathway. Thus, it is impossible to separate effects of AVE0991 on muscle from effects on the RAS system in the tumor and other tissues. Moreover, the experimental design used co-culture of C26 or Lewis Lung Carcinoma (LLC) cells with myotubes, raising the potential that even the in vitro effects of AVE0991 in cachexia conditions might be mediated through signaling on tumor cells. Indeed, the authors do not report muscle or plasma ligand levels to demonstrate that Ang II might be elevated or Ang-(1-7) limiting in cachexia and actually show

that Mas receptor expression is reduced in muscle during cachexia. This leaves open the possibility that while AVE0991 might activate the remaining pool of protein and antagonize local Ang II signaling, it might also act on other organs important in cachexia and known to express Mas, including the brain, heart, vessels, tumor cells, tumor microenvironment, and immune cells (8). Such effects could include central effects on appetite regulation and activity, improvement in cardiovascular function, anti-inflammatory effects, and anti-tumor effects. Moreover, it is possible that the anti-tumor effects can be observed with any effective anti-cachexia therapy; depriving the tumor of a rich source of lipids and amino acids could restrict tumor growth. The authors acknowledge these additional potential mechanisms, particularly the anti-tumor effects of AVE0991 and this sets the stage for further discovery.

The implications of this study are exciting. Given its syndromic nature, the ideal anti-cachexia drug would target multiple drivers of muscle wasting—tumor, inflammation, metabolism—and not necessarily act only at the end-organ or myofiber (9). This might or might not require multi-modal therapy given that known targets in cancer cachexia affect a range of biological processes, e.g. the cytokines Interleukin-6, GDF-15, and Activin (1). In addition, the ideal readout for efficacy is likely a compound metric of symptoms not reducible to a single primary endpoint of lean mass or grip strength (10). As such, AVE0991 or other Mas agonists in clinical development appear to be excellent candidates for testing in patients with cancer cachexia or in patients projected to experience cachexia. With 5-year survival rates for some cachexia-associated cancers including pancreatic cancer and biliary cancers <9%, the bar should be correspondingly low to interrogate such new molecules and mechanisms in rigorously designed interventional trials that account for the systemic, multifactorial nature of this condition.

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